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Drugs; Tests and Methods of Assay and Certification

Title 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER C—DRUGS

PART 147—ANTIBIOTICS INTENDED FOR USE IN THE LABORATORY DIAGNOSIS OF DISEASE

PART 148e—ERYTHROMYCIN

PART 148k—NYSTATIN

PART 148p—POLYMYXIN

PART 148r—TYROTHRICIN

PART 148t—VIOMYCIN

Tests and Methods of Assay and Certification of Antibiotic Drugs Subject to Drug Amendments of 1962

The Commissioner of Food and Drugs has evaluated the views and comments received in response to the notice of proposed rulemaking published in the FEDERAL REGISTER of April 18, 1963 (28 F.R. 3822), and has concluded that the following regulations should issue for the certification of certain antibiotic drugs subject to the Drug Amendments of 1962 (76 Stat. 785–787; Public Law 87–781). Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (21 CFR 2.90; 29 F.R. 471), Title 21 is amended as hereinafter set forth.

§ 147.1 [Amended]

1. Paragraph (e)(1) of § 147.1 *Antibiotic sensitivity discs; tests and methods of assay; potency* is amended by changing the last sentence to read as follows: "This is the potency obtained for a single assay. Perform two or more replicate assays on each of 2 days. The average of all assays is the potency of the sample disc."

2. Section 147.2 is amended as follows: By changing the introduction to paragraph (a), by adding thereto the following new subparagraphs, and by changing (c)(1)(iii), to read as set forth below:

§ 147.2 Antibiotic sensitivity discs; certification procedure.

(a) *Standards of identity, strength, quality, and purity.* Antibiotic sensitivity discs are paper or plastic discs or compressed tablets containing antibiotic compounds. If they are tablets they may contain suitable lubricants, binders, and diluents, none of which shall affect the antibacterial spectrums of the antibiotics. Each disc shall have a uniform potency that is equivalent to that contained in a standard disc prepared with one of the following quantities of antibiotic compounds:

* * * * *

(12) Colistin: Not less than 2 μg or not more than 10 μg.

(13) Erythromycin: Not less than 2 μg or not more than 15 μg.

(14) Kanamycin: Not less than 5 μg or not more than 30 μg.

(15) Neomycin: Not less than 5 μg or not more than 30 μg.

(16) Novobiocin: Not less than 5 μg or not more than 30 μg.

(17) Nystatin: 100 units.

(18) Oleandomycin: Not less than 2 μg or not more than 15 μg.

(19) Oxytetracycline: Not less than 5 μg or not more than 30 μg.

(20) Polymyxin B: Not less than 50 units or not more than 300 units.

(21) Ristocetin: Not less than 5 μg or not more than 30 μg.

(22) Vancomycin: Not less than 5 μg or not more than 30 μg.

(23) Viomycin: Not less than 2 μg or not more than 10 μg.

* * * * *

(c) *Labeling.* * * *

(1) * * *

(iii) The statement "Expiration date ----------," the blank being filled in with the date that is 6 months after the month during which the batch was certified, except that the blank may be filled in with a date that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such longer period of time.

* * * * *

3. Section 147.4 *Antibiotic-dehydrated media-triphenyltetrazolium chloride sensitivity discs* * * * is amended by changing paragraphs (a)(1) and (b) to read:

§ 147.4 Antibiotic-dehydrated media-triphenyltetrazolium chloride sensitivity discs; certification procedure.

(a)(1) Each disc shall contain penicillin G, streptomycin, tetracycline, chloramphenicol, bacitracin, erythromycin, kanamycin, neomycin, novobiocin, oleandomycin, polymyxin B, or ristocetin. If it is tetracycline, it shall contain 5 micrograms.

* * * * *

(b) In all cases, the immediate container shall be sterile at the time of filling and closing. If it is a packaged combination, such package shall contain only those discs described in paragraph (a) of this section.

* * * * *

4. Title 21, Chapter I, is amended by adding thereto the following new sections and parts:

§ 148e.18 Erythromycin ethylsuccinate-butylaminobenzoate intramuscular solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate-butylaminobenzoate intramuscular solution is erythromycin ethylsuccinate and butylaminobenzoate dissolved in polyethylene glycol. It contains a suitable and harmless preservative. Each milliliter contains 50 milligrams of erythromycin. It contains 2 percent butylaminobenzoate. It is sterile. It is nontoxic. Its moisture content is not more than 0.5 percent. The erythromycin ethylsuccinate used conforms to the standards prescribed therefor by § 148e.7(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* (i) In addition to the labeling requirements prescribed by § 148.3 of this chapter, each immediate container shall bear on its label and labeling the statement: "Warning—For intramuscular use only."

(ii) Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, reduce on ignition, identity, and crystallinity.

(*b*) The batch for potency, sterility, toxicity, and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package or immediate container submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3)(ii) (*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b)(1), except prepare the solution of the sample in the following manner: By means of a syringe, transfer 1 milliliter of the sample to a 100-milliliter volumetric flask, dissolve with 40 milliliters of absolute methyl alcohol, fill to volume and further dilute to the reference point with 0.1*M* potassium phosphate buffer, pH 8.0. The erythromycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the amount that it is represented to contain.

(2) *Sterility.* Use the entire contents of a single-dose container, or approximately 1 milliliter from each multiple-dose container and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except administer subcutaneously a test dose of 0.1 milliliter of the undiluted solution.

(4) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter, except dissolve the sample with absolute methyl alcohol.

§ 148e.23 Erythromycin ethyl carbonate for pediatric drops.

(a) *Requirements for certification—*(1) *Standards of identity, strength, qual-*

ity, and purity. Erythromycin ethyl carbonate for pediatric drops is a dry mixture of erythromycin ethyl carbonate, suitable and harmless dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains 100 milligrams of erythromycin. Its moisture content is not more than 2.0 percent. Its pH is not less than 6.5 nor more than 7.0. The erythromycin ethyl carbonate used conforms to the standards prescribed by § 148e.2(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethyl carbonate used in making the batch for potency, toxicity, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin ethyl carbonate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each immediate container or package submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.11 (b)(1). The erythromycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using 500 milligrams.

(3) *pH.* Proceed as directed in § 141a.5 (b) of this chapter, using the suspension reconstituted as directed in the labeling.

PART 148k—NYSTATIN

Sec.

148k.1 Nystatin.
148k.2 Nystatin ointment.
148k.3 Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment.
148k.4 Nystatin topical powder.
148k.5 Nystatin-neomycin sulfate-gramicidin topical powder.
148k.6 Nystatin for oral suspension.
148k.7 Nystatin tablets.
148k.8 Nystatin cream.
148k.9 Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.
148k.10 Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution.
148k.11 Nystatin vaginal tablets.

AUTHORITY: The provisions of this Part 148k issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C. 357.

§ 148k.1 Nystatin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin is the yellow to light-tan compound of a kind of nystatin or a mixture of two or more such compounds. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. It is so purified and dried that:

(i) Its potency is not less than 2,000 units of nystatin per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH in a 3 percent aqueous suspension is not less than 6.5 and not more than 8.0.

(v) It exhibits absorption maxima within ±2 millimicrons at 230 millimicrons, 291 millimicrons, 305 millimicrons, and 319 millimicrons when dissolved in methyl alcohol, and the ratio of the absorption peak at 230 millimicrons ±2 millimicrons and the middle of the shoulder at 279 millimicrons ±2 millimicrons, $\frac{A_{230}}{A_{279}}$, is not less than 0.90 and not more than 1.25.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for carrying the organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) Make nutrient agar for preparing the inoculated agar plates, as follows:

Peptone	9.4 gm.
Yeast extract	4.7 gm.
Beef extract	2.4 gm.
Sodium chloride	10.0 gm.
Dextrose	10.0 gm.
Agar	23.5 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.0 to 6.2 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard.* Dry an appropriate amount of the working standard for 2 hours at 40° C. at a pressure of 5 millimeters or less. Determine the dry weight and dissolve in sufficient dimethyl formamide to give a stock solution of convenient concentration (usually 1,000 units to 5,000 units per milliliter). This stock solution should be prepared simultaneously with the samples to be tested and should be used for 1 day only.

(*d*) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethylformamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated).

(*e*) *Preparation of test organism.* The test organism is Saccharomyces cerevisiae (ATCC 2601, no vitamin requirement), which is maintained on slants of agar described in (*b*)(*1*) of this subdivision. Wash the organism from the agar slant with 3 milliliters of sterile distilled water onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (*b*)(*1*) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. and then wash the resulting growth from the agar surface with about 30 millliliters of sterile distilled water. Standardize the suspension so that a 1:30 dilution of it will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the adjusted stock suspension (not the 1:30 dilution) that should be added (usually 0.7 milliliter) to each 100 milliliters of agar to give clear, sharp inhibition zones of appropriate size.

(*f*) *Preparation of plates.* Melt a sufficient amount of the agar described in (*b*)(*2*) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in (*e*) of this subdivision, and mix thoroughly. Add 8 milliliters of this inoculated agar to each Petri dish (100 millimeters x 20 millimeters, with flat bottom). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Dilute aliquots of the standard stock solution with dimethylformamide to give concentrations of 256, 320, 400, 500, and 624 units of nystatin per milliliter. Dilute these solutions with 10 percent potassium phosphate buffer, pH 6.0, to make concentrations of 12.8, 16.0, 20.0, 25.0, and 31.2 units

per milliliter, respectively. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 20 units per milliliter, solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 20 units per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be 36 twenty-unit determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 30° C. and measure the diameter of each zone of inhibition. Average the readings of the 20 units per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 20 units per milliliter concentration. The average of the 36 readings of the 20 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 20 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 16 units per milliliter concentration, the average of the 36 readings of the 20 units per milliliter concentration is 16.0 millimeters and the average of the 20 units per milliliter concentration of this set of three plates is 15.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 16 units per milliliter concentration of those same three plates is 15.0 millimeters, the corrected value is then 15.2 millimeters. Plot these corrected values, including the average of the 20 units per milliliter concentration, on two-cycle semilog paper, using the concentration in units per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 20 units per milliliter standard;

a, b, d, e = Corrected average values for the 12.8, 16.0, 25.0, and 31.2 units per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 20 units per milliliter standard and three cylinders with the 20 units per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 30° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 20 units per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 20 units per milliliter value on the curve. From the curves, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay—(a) Culture medium.* Make nutrient broth for preparing an inoculum of the test organism as follows:

Peptone	10 gm.
Dextrose	20 gm.
Distilled water, q.s.	1,000 ml.

pH 5.6 to 5.7 after sterilization.

In lieu of preparing the medium from the individual ingredients specified in this subdivision, it may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified in this subdivision are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(*b*) *Working standard and standard solutions.* Prepare the standard stock solution as directed in subdivision (i) (*c*) of this subparagraph. Make dilutions of the stock solution with dimethylformamide to obtain concentrations of 121, 139, 184, and 212 units per milliliter. These solutions are then diluted 1:10 with sterile distilled water to obtain concentrations of 12.1, 13.9, 16.0, 18.4, and 21.2 units per milliliter. Add 1.0 milliliter of each such concentration to each of six sterile 20 millimeters x 150 millimeters test tubes. Maintain the sterility of the tubes.

(*c*) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethylformamide to give a stock solution of convenient concentration. Dilute the stock solution with dimethylformamide to obtain a concentration of 160 units per milliliter (estimated). Further dilute this solution 1:10 with sterile distilled water to obtain a concentration of 16 units per milliliter (estimated). Add 1.0 milliliter of this dilution to each of six sterile 20 millimeters x 150 millimeters test tubes.

(*d*) *Preparation of test organism.* Maintain the test organism Saccharomyces cerevisiae (ATCC 9763) on agar slants as described in subdivision (i) (*e*) of this subparagraph. For use in the assay, prepare an organism suspension by inoculating 100 milliliters of the medium described in (*a*) of this subdivision with a loopful of growth from an agar slant, and incubate the broth culture overnight at 37° C. Adjust this stock suspension, so that a 1:30 dilution in the nutrient broth will give 25 percent light transmission at a wavelength of 580 millimicrons, using a suitable photoelectric colorimeter and a 13-millimeter diameter test tube as an absorption cell. Prepare the inoculum broth by adding from 3 milliliters to 5 milliliters of that adjusted suspension (not the 1:30 dilution) to each liter of nutrient broth needed for the test.

(*e*) *Procedure.* To each of the 20 millimeters x 150 millimeters test tubes prepared in (*b*) and (*c*) of this subdivision, add 9.0 milliliters of the inoculated nutrient broth described in (*d*) of this subdivision, and incubate at 26° C. for 16 hours to 18 hours. After incubation, add 0.5 milliliter of a 12 percent formaldehyde solution to each tube and read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in (*a*) of this subdivision.

(*f*) *Estimation of potency.* Plot the average values for each concentration of the standard on semilog graph paper with absorbance values on the arithmetic scale and nystatin concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated absorbance value for the lowest concentration of the standard curve;

H = Calculated absorbance value for the highest concentration of the standard curve;

a, b, c, d, e = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance values for the sample and read the nystatin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the nystatin content of the sample.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject intraperitoneally each of 5 mice with 0.5 milliliter of a suspension of the drug containing 1,200 units per milliliter in a 0.5 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 3 percent aqueous suspension of the drug.

(5) *Identity.* Weigh approximately 20 milligrams of the sample in a 100-milliliter glass-stoppered volumetric flask, add about 75 milliliters absolute methyl alcohol and shake mechanically for 30 minutes. Dilute to volume with methyl alcohol. Transfer 10.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Within 2 hours, determine the absorption peak at 230 millimicrons, 291 millimicrons, 305 millimicrons, and 319 millimicrons, and the shoulder at 279±2 millimicrons, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with absolute methyl alcohol. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 millimicrons to 320 millimicrons. If a non-

recording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two $\left(\text{absorbances } \frac{A_{230}}{A_{279}}\right)$ should be not less than 0.90 and not more than 1.25.

§ 148k.2 Nystatin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin ointment is composed of nystatin and a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin. The moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, one package of each other ingredient used containing 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Using sufficient dimethylformamide to give an estimated concentration of 400 nystatin units per milliliter, blend an accurately weighed representative portion (usually 1 gram) in a high-speed glass blender for 3 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units of nystatin per milliliter, and proceed as directed in § 148k.1(b)(1). Its nystatin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148k.3 Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidintriamcinolone acetonide ointment is nystatin, neomycin sulfate, gramicidin, and triamcinolone acetonide, in a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1 milligram of triamcinolone acetonide. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (v), (vi), and (vii) of this chapter. The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of 7 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3)(ii)(*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 148k.2(b)(1). Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin per gram that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.3(b)(1) of this chapter. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin per gram that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except prepare the sample in the following manner: Accurately weigh and dissolve approximately 2 grams of the sample in 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent ethyl alcohol. Repeat the extraction three times. Pool the extractives and bring to a volume of 100 milliliters with 80 percent ethyl alcohol. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148k.4 Nystatin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin topical powder is a dry powder composed of nystatin and talc. Each gram contains 100,000 units of nystatin. Its moisture content is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used: One container of each consisting of not less than 5 grams.

(4) Fees. $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Blend the entire contents of an accurately weighed representative sample for 2 to 3 minutes in a high-speed, glass blender with 500 milliliters of dimethylformamide. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter. Further dilute in 10 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 148k.1(b)(1). The potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148k.5 Nystatin-neomycin sulfate-gramicidin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin topical powder is a dry powder composed of nystatin, neomycin sulfate, gramicidin, and talc. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, and

0.25 milligram of gramicidin. Its moisture content is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 418k.1(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by §148i.1(a)(1) (i), (v), (vi), and (vii) of this chapter. The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, PH, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, and identity.

(*d*) The batch for nystatin content, neomycin content, gramicidin content, and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of 7 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3)(ii)(*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 148k.4(b)(1). The nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin per gram that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.1(b)(1) of this chapter, except prepare the sample in the following manner: Blend an accurately weighed representative sample in 500 milliliters of 0.1*M* postassium phosphate buffer, pH 8.0. Further dilute an aliquot to the reference concentration with 0.1*M* potassium phosphate buffer, pH 8.0. The neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of nystatin per gram that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except prepare the sample in the following manner: Dissolve the entire contents or a 1-gram sample in 95 percent ethyl alcohol, and filter. Collect the filtrate, and dilute a portion to the reference concentration with 95 percent ethyl alcohol. The gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148k.6 Nystatin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin for oral suspension is a dry powder consisting of nystatin, and suitable and harmless suspending substances, preservatives, diluents, colorings, and flavorings. When the suspension is prepared as directed in its labeling each milliliter contains 100,000 units of nystatin. The pH of the reconstituted drug is not less than 4.9 and not more than 5.5. Its moisture content is not more than 7.0 percent. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used: One container of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the drug as directed in the labeling. Blend an appropriate aliquot (usually from 1 milliliter to 5 milliliters) in a high-speed, glass blender for 2 to 3 minutes, using 200 milliliters of dimethylformamide. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Proceed as directed in § 148k.1(b)(1). Its potency is satisfactory if it contains not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Using the dry powder, proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension after reconstituting as directed in the labeling.

§ 148k.7 Nystatin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin tablets are tablets composed of nystatin and suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 500,000 units of nystatin. The moisture content is not more than 5 percent. The tablets shall distintegrate within 2 hours. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(2) For disintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used: One container of each consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(2) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Blend a representative number of tablets for 2 to 3 minutes in a high-speed glass blender with 500 milliliters of dimethylformamide. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot in 10 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 148k.1(b)(1). The potency is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148k.8 Nystatin cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, qual-*

ity, and purity. Nystatin cream is composed of nystatin and suitable and harmless emulsifiers, perfumes, buffers, preservatives, and a protectant, in a suitable and harmless cream base. Each gram contains 100,000 units of nystatin. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay; potency.* Using sufficient dimethylformamide to give an estimated concentration of 400 units of nystatin per milliliter, blend an accurately weighed representative portion (usually 1 gram) in a high-speed blender for 3 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 to an estimated concentration of 20 units of nystatin per milliliter, and proceed as directed in § 148k.1(b)(1). Its nystatin content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of units of nystatin that it is represented to contain.

§ 148k.9 Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream is composed of nystatin, neomycin sulfate, gramicidin, triamcinolone acetonide, and suitable and harmless emulsifiers, solvents, perfumes, buffers, preservatives, and a protectant, in a suitable cream base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1 milligram of triamcinolone acetonide. The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (v), (vi), and (vii) of this chapter. The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* (i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, and identity.

(*d*) The batch for nystatin content, neomycin content, and gramicidin content.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of 7 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*d*) of this paragraph; $6.00 for each package submitted in accordance with subparagraph (3)(ii)(*e*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Nystatin content.* Proceed as directed in § 148k.2(b)(1). The nystatin content is satisfactory if it is not less than 90 percent nor more than 140 percent of the number of units of nystatin that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.3(b)(1) of this chapter. The neomycin content is satisfactory if it is not less than 90 percent nor more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(iii) *Gramicidin.* Proceed as directed in § 148f.1(b)(1) of this chapter, except prepare the sample in the following manner: Using 499 milliliters of 95 percent ethyl alcohol and 1 milliliter of polysorbate 80, blend an accurately weighed representative portion, usually 2 grams, in a high-speed glass blender. Further dilute with 95 percent ethyl alcohol to the reference concentration. The gramicidin content is satisfactory if it is not less than 90 percent nor more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

§ 148k.10 Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-polymyxin B sulfate-fludrocortisone acetate for otic solution is a dry mixture of nystatin, neomycin sulfate, polymyxin B sulfate, and fludrocortisone acetate. Its moisture content is not more than 6.5 percent. It may be packaged in combination with an immediate container of a suitable and harmless aqueous diluent, containing suitable preservatives and buffers. When reconstituted as directed in the labeling, each milliliter contains 100,000 units of nystatin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 1 milligram of fludrocortisone acetate. Its pH is not less than 4.0 and not more than 6.0. They nystatin used conforms to the standards prescribed by § 148k.1(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (v), (vi), and (vii) of this chapter. The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, moisture, residue on ignition, pH, and identity.

(*d*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*c*) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*d*) The batch: A minimum of 7 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3)(ii)(*d*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Nystatin.* Proceed as directed in § 148k.6(b)(1). Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.1(b)(1) of this chapter, using the suspension obtained after

reconstituting as directed in the labeling. The neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(iii) *Polymyxin B content.* Use the suspension obtained after reconstituting as directed in the labeling. Proceed as directed in § 148p.1(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. The polymyxin B content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the drug reconstituted as directed in its labeling.

§ 148k.11 Nystatin vaginal tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Nystatin vaginal tablets are tablets composed of nystatin and suitable and harmless diluents, binders, and lubricants. Each tablet contains 100,000 units of nystatin. The moisture content is not more than 5 percent. The disintegration time is not more than 1 hour. The nystatin used conforms to the standards prescribed therefor by § 148k.1(a)(1) (i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for nystatin content, moisture,, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 immediate containers of approximately 300 milligrams each.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148k.-7(b)(1). The nystatin content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter, using distilled water as the immersion fluid.

PART 148p—POLYMYXIN

Sec.

148p.1 Polymyxin B sulfate.
148p.2 Polymyxin B sulfate tablets.
148p.3 Polymyxin B sulfate ointment.
148p.4 Polymyxin B sulfate otic solution.
148p.5 [Reserved]
148p.6 Polymyxin B sulfate soluble tablets.

AUTHORITY: The provision of this Part 148p issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148p.1 Polymyxin B sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its residue on ignition is not more than 5 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 100 parts per million.

(ix) It gives positive color identity tests for polymyxin.

(2) *Packaging.* In addition to the requirements of § 148.2 of this chapter, if it is packaged for dispensing and it is intended for intrathecal, intramuscular, or topical use, each immediate container shall contain 500,000 units.

(3) *Labeling.* In addition to the requirements of § 148.3 of this chapter, if the drug is packaged for dispensing its labeling shall bear the statement, "Caution this drug should be given intramuscularly and/or intrathecally only to hospitalized patients so as to provide constant supervision by a physician."

(4) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, residue on ignition, heavy metals, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 10 packages, each containing approximately 300 milligrams.

(*b*) If the drug is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers plus one additional package containing 1 gram of the batch.

(*2*) For sterility testing: 10 immediate containers.

(5) *Fees.* $5.00 for each package or immediate container submitted in accordance with subparagraph (4)(ii) (*a*) (*1*) and (*b*)(*1*) of this paragraph; $10.00 for all packages or immediate containers submitted in accordance with subparagraph (4)(ii) (*a*)(*2*) and (*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Using ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F., make nutrient agar for carrying the test organism and for the seed and base layers:

(*a*) For carrying the test organism:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*b*) Base layer:

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Agar	20.0 gm.
Distilled water, q.s.	1,000.0 ml.

Final pH 7.3 after sterilization.

(*c*) Seed layer: For the seed layer, make the following changes in the medium described in (*b*) of this subdivision.

Agar	12.0 gm.
Polysorbate 80 (added after boiling)	10.0 gm.

Final pH 7.3 after sterilization.

In lieu of preparing the median from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such median. Minor modifications of the specified individual ingredients are permissible if the resulting median possesses growth-promoting properties at least equal to the median described.

(iii) *Working standard.* Dry approximately 30 milligrams of the standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine accurately the dry weight, dissolve in 2.0 milliliters of distilled water, and then add sufficient 10 percent potassium phosphate buffer, pH 6.0, to make a 1,000 units per milliliter stock solution. This

solution may be used for 2 weeks if kept in refrigerator.

(iv) *Preparation of sample.* Dissolve an accurately weighed sample in 2 milliliters of distilled water and dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration; and, if it is packaged for dispensing, reconstitute as directed in the labeling, remove an accurately measured representative portion with a suitable syringe fitted with a 22-gage needle, and appropriately dilute in 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute in 10 percent potassium phosphate buffer, pH 6.0, to give a final estimated concentration of 10 units per milliliter of polymyxin.

(v) *Preparation of test organism.* The test organism is Bordetella bronchiseptica (ATCC 4617), which is maintained on slants of agar described in subdivision (ii) (*a*) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large surface such as that provided by a Roux bottle containing 300 milliliters of agar described in subdivision (ii) (*a*) of this subparagraph. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. Standardize the suspension, so that a 1:20 dilution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the 1:20 diluted suspension (usually 0.3 percent) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*b*) of this subparagraph to each Petri dish (20 millimeters by 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow it to harden. Melt a sufficient amount of the agar described in subdivision (ii) (*c*) of this subparagraph, cool to 48° C.–50° C., and the proper amount of test organism as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 units per milliliter working standard stock solution in 10 percent potassium phosphate buffer, pH 6.0, to obtain concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter. Use three plates for the determination of each point on the curve, except the 10.0 units per milliliter concentration, a total of 12 plates. On each of three plates, fill three cylinders with the 10.0 units per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 10.0-unit determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 10.0 units per milliliter and the readings of the points tested for each set of three plates, and average also all 36 readings of the 10.0 units per milliliter concentration. The average of the 36 readings of the 10.0 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 10 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 8.0 units per milliliter concentration, the average of the 36 readings of the 10-unit concentration is 20.0 millimeters and the average for the 10-unit concentration of this set of three plates is 19.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 8.0-unit concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 10 units per milliliter concentration, on semilog paper, placing the concentration in units per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter of the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 10 units per milliliter standard;

a, b, d, e = Corrected values for the 6.4, 8.0, 12.5, and 15.6 units per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 10 units per milliliter standard solution and three cylinders with the 10 units per milliliter (estimated) sample, alternating sample and standard. Incubate all plates, including those containing the standard curve, for 16 hours to 18 hours at 37° C. Measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 10 units per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 10 units per milliliter value on the curve. Read the potencies corresponding to these corrected values of zone size. If packaged for dispensing, its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of units of polymyxin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 141a.2 of this chapter using 50 milligrams from each sample tested, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, except use as a test dose 1.0 milliliter per kilogram of solution containing 20,000 units per milliliter in sterile, pyrogen-free U.S.P. saline T.S.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution containing 1,200 units per milliliter in sterile U.S.P. saline T.S.

(5) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, except use a solution containing 5 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(8) *Heavy metals content.* Proceed as directed in § 148m.1(b)(9) of this chapter.

(9) *Identity.* (i) To a solution of 2 milligrams of polymyxin sulfate in 5 milliliters of water add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops.

(ii) To a solution of 2 milligrams of polymyxin sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop; a reddish-violet color is produced.

§ 148p.2 Polymyxin B sulfate tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate tablets are tablets that contain polymyxin B sulfate, with one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains 500,000 units of polymyxin B. The moisture content is not more than 7.0 percent. They shall disintegrate within 30 minutes. The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (iv), (v), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, toxicity,

moisture, pH, residue on ignition, and identity.

(b) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(a) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except disintegration time: Minimum of 30 tablets.

(2) For disintegration time: Six tablets.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3) (ii) (b) (1) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3) (ii) (b) (2) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1 (b) (1), except prepare the sample by blending a representative number of tablets in a high-speed glass blender with an appropriate amount of 10 percent potassium phosphate buffer, pH 6.0, to prepare a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot in 10 percent potassium phosphate buffer, pH 6.0, to a final estimated concentration of 10 units of polymyxin B per milliliter. The potency is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148p.3 Polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate ointment is polymyxin B sulfate in a suitable and harmless ointment base. Its potency is 20,000 units per gram. Its moisture content is not more than 1.0 percent. The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a) (1) (i), (v), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1 (b) (1), except prepare the sample by accurately weighing a representative portion of the ointment (usually 1.0 gram) and transferring it to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake with four 25-milliliter portions of 10 percent potassium phosphate buffer, pH 6.0, and combine the aqueous extractives. After adjusting the volume of the combined extractives to 100 milliliters with 10 percent potassium phosphate buffer, pH 6.0, remove an aliquot and further dilute with 10 percent potassium phosphate buffer, pH 6.0, to obtain a final estimated concentration of 10 units of polymyxin B per milliliter. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of units per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148p.4 Polymyxin B sulfate otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate otic solution is polymyxin B sulfate with or without one or more suitable and harmless preservatives and buffer substances, in a suitable and harmless vehicle. Each milliliter contains 10,000 units of polymyxin B. It may contain 5.0 milligrams of hydrocortisone per milliliter. It is sterile. If it contains acetic acid, its pH is not less than 3.8 and not more than 4.3. The polymyxin B sulfate used conforms to the standards of § 148.1(a) (1) (i), (v), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(b) The batch for potency, sterility, and, if it contains acetic acid, pH.

(ii) Samples required:

(a) Polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 5 immediate containers.

(2) For sterility testing: 10 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (a) and (b) (1) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (b) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1 (b) (1), except prepare the sample by diluting volumetrically a representative portion (usually 1.0 milliliter) in 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute an aliquot in 10 percent potassium phosphate buffer, pH 6.0, to give a final estimated concentration of 10 units of polymyxin B per milliliter. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Use 0.25 milliliter from each immediate container and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

§ 148p.5 [Reserved]

§ 148p.6 Polymyxin B sulfate soluble tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate soluble tablets are tablets composed of polymyxin B sulfate and one or more suitable and harmless binders and fillers. Each tablet contains 250,000 units of polymyxin B. The moisture content is not more than 5.0 percent. The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1 (i), (iv), (v), (vi), (vii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The polymyxin B sulfate used in making the batch for potency, toxicity, moisture, pH, residue on ignition, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 tablets.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(b) of this paragraph; $4.00 for each package submitted in accordance with subparagraph (3)(ii) (a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1(b)(1), except prepare the sample for assay by placing a representative number of tablets into a volumetric flask of appropriate size. Swirl the tablets in a quantity of distilled water just sufficient to dissolve them. Adjust to volume with 10 percent potassium phosphate buffer, pH 6.0. Further dilute an aliquot to a final estimated concentration of 10 units of polymyxin B per milliliter in 10 percent potassium phosphate buffer, pH 6.0. The potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

PART 148r—TYROTHRICIN

§ 148r.1 Tyrothricin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tyrothricin is a white to brownish-white compound of a kind of tyrothricin or a mixture of two or more such compounds. It consists principally of gramicidin and tyrocidine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,400 micrograms of tyrothricin per milligram.

(ii) Its moisture content is not more than 5 percent.

(iii) It gives a positive identity test for tyrothricin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and identity.

(ii) Samples required from each batch: 5 packages, each containing approximately 300 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Tyrothricin is assayed using the gramicidin working standard. Proceed as directed in § 148f.1(b)(1) of this chapter, except dilute the tyrothricin sample to a final estimated concentration of 0.20 microgram of tyrothricin per milliliter. Average the absorbance values for the tyrothricin sample, and read the gramicidin concentration from the gramicidin standard curve. Multiply by 5 to obtain the number of micrograms of tyrothricin in the sample.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Identity.* To 5 milliliters of *p*-dimethylaminobenzaldehyde (T.S.) add about 5 milligrams of tyrothricin, shake well for 2 minutes, then add 2 drops of a 0.1*M* sodium nitrite and 5 milliliters of water. A blue color is produced.

(Sec. 507, 59 Stat. 463, as amended, 76 Stat. 785, 786, 787; 21 U.S.C.A. 357)

PART 148t—VIOMYCIN

§ 148t.1 Viomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Viomycin sulfate is the sulfate salt of viomycin. It is so purified and dried that:

(i) Its potency is not less than 700 micrograms of viomycin per milligram, on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5 percent.

(vii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) Its absorption maximum at approximately 268 $m\mu$ does not vary more than ±3 millimicrons from that of the viomycin working standard, similarly treated.

(2) *Packaging.* In addition to the requirements of § 148.2 of this chapter, if it is packaged for dispensing the viomycin content shall be 1 gram or 5 grams of viomycin per immediate container.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(4) *Request for certification; sample.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, and identity.

(ii) Samples required:

(a) If the batch is packaged for repacking or for use in the manufacture of another drug:

(1) For all tests except sterility: A minimum of 10 packages, each containing 300 milligrams.

(2) For sterility: 10 packages, each containing 300 milligrams.

(b) If the batch is packaged for dispensing:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility: 10 immediate containers.

(5) *Fees.* $5.00 for each container in the sample submitted in accordance with subparagraph (4)(ii) (a)(1) or (b)(1) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (4)(ii) (a)(2) or (b)(2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay*—(a) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(b) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) Make nutrient agar for preparing the inoculated plates as follows:

Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Peptone	6.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in (b) of this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified in (b) of this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(c) *Working standard.* Dry approximately 30 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve in sufficient distilled water to obtain a stock standard solution of 1,000 micrograms per milliliter. Keep this stock solution in a refrigerator. Do not use it later than 1 week after it is made.

(d) *Preparation of sample.* Dissolve the sample to be tested in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 50 micrograms of viomycin per milliliter (estimated).

(e) *Preparation of test organism.* The test organism is Bacillus subtilis (ATCC 6633). Maintain the test organism on nutrient agar prepared as described in (b)(1) of this subdivision. Prepare a spore suspension by one of the following methods:

(1) Grow the organism for 1 week at 37° C. in a number of Roux bottles, each containing 300 milliliters of nutrient agar prepared as described in (b)(1) of this subdivision. Suspend the spores in sterile distilled water, and heat for 30 minutes at 70° C. Wash the spore suspension three times with sterile distilled water, heat again for 30 minutes at 70° C., and resuspend in sterile distilled water; or

(2) Grow the organism for 5 days at 37° C. in a Roux bottle containing 300 milliliters of the agar medium described in (b)(1) of this subdivision, but modified by the addition of 300 milligrams of $MnSO_4 \cdot H_2O$ per liter. Suspend the growth in 50 milliliters of sterile U.S.P. saline T.S., centrifuge, and decant the

supernatant liquid. Reconstitute the sediment and heat-shock the suspension by heating for 30 minutes at 70° C. Maintain the spore suspension at approximately 15° C. Determine by appropriate tests the quantity of spore suspension to be added to each 100 milliliters of agar for the secondary layer that will give sharp, clear zones of inhibition.

(*f*) *Preparation of plates.* Add 10 milliliters of melted agar prepared as described in (*b*) (*2*) of this subdivision to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed only on the outside, and allow to harden. Use the plates the same day they are prepared. Melt the agar to be used for the second layer, cool to 55° C. to 60° C., and add the spore suspension prepared as described in (*e*) of this subdivision. Mix thoroughly, and add 4 milliliters to each of the plates containing the 10 milliliters of uninoculated agar. Tilt the plates back and forth to spread the inoculated agar evenly over the surface. Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer (pH 8.0) to obtain concentrations of 32.0, 40.0, 50.0, 62.5, and 78.1 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 50.0 micrograms per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 50.0 micrograms per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 50-microgram determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 50.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 50.0 micrograms per milliliter concentration. The average of the 36 readings of the 50.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 50.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 40.0 microgram concentration, the average of the 36 readings of the 50.0 microgram concentration were 20.0 millimeters, and the average of the 50.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 40.0-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 50.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentrations in micrograms per milliliter as the ordinate (logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 50.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for the 32.0, 40.0, 62.5, and 78.1 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H, and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 50 micrograms per milliliter solution and three cylinders with the 50.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve at 37° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 50.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 50.0 micrograms per milliliter value on the curve, and read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay*—(*a*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for carrying the organism, as follows:

Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Peptone	6.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(*2*) Make nutrient broth for preparing an inoculum of the test organism as follows:

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

In lieu of preparing the media from the individual ingredient specified in this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*b*) *Test culture.* Employ the agar described in (*a*) (*1*) of this subdivision for maintaining the test organism which is Klebsiella pneumoniae (ATCC 10031), noncapsulated. Transfer stock cultures every week for test purposes. Transfer the organism to fresh agar slants and incubate overnight at 37° C. Suspend the growth from two or three of these slants in sterile U.S.P. saline T.S., and add approximately 5 milliliters of culture suspension to each of two Roux bottles containing the agar described in (*a*) (*1*) of this subdivision. Incubate the bottles overnight at 37° C., harvest the growth using 50 milliliters of sterile U.S.P. saline T.S. per bottle, and pool the washings from the two bottles. Determine the dilution with sterile U.S.P. saline T.S. that will give a light transmission reading of 25 percent, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Keep the resulting suspension of organisms in the refrigerator and use for a period not to exceed 2 weeks. Prepare the daily inoculum by adding 0.1 milliliter–0.2 milliliter of the suspension to each 100 milliliters of the nutrient broth prepared as directed in (*a*) (*2*) of this subdivision, cooled to a temperature of approximately 15° C.

(*c*) *Working standard and standard curve.* To prepare solutions for the standard curve, dilute the 1,000 micrograms per milliliter working standard described in (i) (*c*) of this subparagraph in distilled water to make concentrations of 64, 80, 100, 125, and 156 micrograms of viomycin per milliliter. The 100 micrograms per milliliter concentration is the reference point of the curve. Add 1.0 milliliter of each dilution to each of three test tubes (16 millimeters x 125 millimeters).

(*d*) *Preparation of sample.* Dilute the sample under test in sterile distilled water to contain 100 micrograms per milliliter (estimated). Add 1.0 milliliter of this dilution to each of three test tubes (16 millimeters x 125 millimeters).

(*e*) *Procedure.* To each of the tubes prepared as described in (*c*) and (*d*) of this subdivision, add 9.0 milliliters of the inoculated broth prepared as outlined in (*b*) of this subdivision. Place all tubes immediately in a water bath at 37° C. for 3 hours to 4 hours. After incubation, add 0.5 milliliter of 12 percent formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument to zero absorbance with clear, uninoculated broth.

(*f*) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on two-cycle semilogarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Calculated absorbance value for the lowest concentration of the standard curve;

H=Calculated absorbance value for the highest concentration of the standard curve;

a, b, c, d, e=Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H, and connect the points with a straight line. Average the absorbance value for the sample and read the viomycin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the viomycin content of the sample.

When packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent nor more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Using 300 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Using as a test dose 1.0 milliliter per kilogram of a sterile, pyrogen-free U.S.P. saline test solution containing 10 milligrams of viomycin per milliliter, proceed as directed in § 141a.3 of this chapter.

(4) *Toxicity.* Using as a test dose 0.5 milliliter of a sterile U.S.P. saline test solution, containing 2 milligrams of viomycin per milliliter, proceed as directed in § 141a.4 of this chapter.

(5) *Histamine.* Using as a test dose 0.6 milliliter per kilogram of a sterile U.S.P. saline test solution containing 5 milligrams of viomycin per milliliter, proceed as directed in § 141b.105 of this chapter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 100 milligrams of viomycin per milliliter.

(8) *Identity*—(i) *Phosphate buffer solution.* Mix 296.3 milliliters of 0.1*N* sodium hydroxide with 500 milliliters of 0.05*M* postassium phosphate (6.805 grams of KH_2PO_4 per 1,000 milliliters of water) and dilute with sufficient water to make 1 liter.

(ii) *Procedure.* Dissolve the working standard and the sample to be tested in sufficient potassium phosphate buffer solution to give solutions containing 10 micrograms per milliliter. Measure the ultraviolet absorption of each solution in a suitable spectrophotometer. Compare the spectrum of the sample qualitatively with that of the standard.

Effective date. This order shall become effective 60 days from the date of its publication in the FEDERAL REGISTER.

(Sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357)

Dated: June 15, 1964.

JOHN L. HARVEY,
Deputy Commissioner of Food and Drugs.

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